EXHIBIT A: MARKED VERSION OF AMENDED PARAGRAPHS IN THE SPECIFICATION

U.S. APPLICATION SERIAL NO. 09/616,849
(ATTORNEY DOCKET NO. 9301-044)

(as amended October 9, 2001)

On page 1, please amend the paragraph beginning "Within the past decade", as follows:

Within the past decade, several technologies have made it possible to monitor the expression level of a large number of genetic transcripts at any one time (see, *e.g.*, Schena *et al.*, 1995, *Science 270*:467-470; Lockhart *et al.*, 1996, *Nature Biotechnology 14*:1675-1680; Blanchard *et al.*, 1996, *Nature Biotechnology 14*:1649; Ashby *et al.*, U.S. Patent No. 5,569,588, issued October 29, 1996). For example, techniques are known for preparing microarrys of cDNA transcripts (see, *e.g.*, DeRisi *et al.*, 1996, *Nature Genetics 14*:457-460; Shalon *et al.*, 1996, *Genome Res. 6*:689-645; and Schena *et al.*, 1995, *Proc. Natl. Acad. Sci. U.S.A. 93*:10539-11286). Alternatively, high-density arrays containing thousand of oligonucleotides complementary to defined sequences, at defined locations on a surface using photolithographic techniques for synthesis *in situ* are described, *e.g.*, Fodor *et al.*, 1991, *Science 251*:767-773; Pease *et al.*, 1994, *Proc. Natl. Acad. Sci. U.S.A. 91*:5022-5026; Lockhart *et al.*, 1996, *Nature Biotechnology 14*:1675; U.S. Patent Nos. 5,578,832; 5,556,752; and 5,510,270). Methods for generating arrays using inkjet technology for oligonucleotide synthesis are also known in the art (see, *e.g.*, Blanchard, International Patent Publication WO 98/41531, published September 24, 1998; Blanchard *et al.*, 1996, *Biosensors and [Bioeletronics]* *Bioelectronics* *11*:687-690; Blanchard, 1998, in *Synthetic DNA Arrays in Genetic Engineering*, Vol. 20, J.K. Setlow, Ed., Plenum Press, New York at pages 111-123).

On page 5, please amend the paragraph beginning "The methods of the invention are particularly", as follows:

The methods of the invention are particularly useful for evaluating large numbers of different probes. For example, the methods of the invention can be used to evaluate a plurality of different probes by comparing the amount or number of molecules from a first sample (*i.e.*, a specific binding sample) that bind to a each of the plurality of different probes

to the number or amount of molecules from a second sample (*i.e.*, a non-specific binding sample) that bind to each of the plurality of different probes. In preferred embodiments, the methods of the invention are used to evaluate a plurality of different probes in an array of probes, wherein the array comprises a solid (or, in certain embodiments, semi-solid) support or surface to which molecules of the plurality of different probes are immobilized. Most preferably, the array is an addressable array, such as a positionally addressable array wherein each different probe is located at a specific, known location on the support or surface such that the [identitiy] <u>identity</u> of a particular probe can be determined from its location on the support or surface.

On page 16, please amend the paragraph beginning "Preferably, the detectable label is a fluorescent label", as follows:

Preferably, the detectable label is a fluorescent label, *e.g.*, by incorporation of nucleotide analogs. Other labels suitable for use in the present invention include, but are not limited to, biotin, imminobiotin, antigens, cofactors, dinitrophenol, lipoic acid, olefinic compounds, detectable polypeptides, electron rich molecules, enzymes capable of generating a detectable signal by action upon a substrate, and radioactive isotopes. Preferred radioactive isotopes include, ^{32}P, ^{35}S, ^{14}C, ^{15}N and ^{125}I, to name a few. Fluorescent molecules suitable for the present invention include, but are not limited to, fluorescein and its derivatives, rhodamine and its derivatives, texas red, 5'-carboxy-fluorescein ("FMA"), 2',7'-dimethoxy-4',5'-dichloro-6-carboxy-fluorescein ("JOE"), N,N,N',N'-tetramethyl-6-carboxy-rhodamine ("TAMRA"), 6'-carboxy-X-rhodamine ("ROX"), HEX, TET, IRD40 and IRD41. Fluroescent molecules that are suitable for the invention further include: cyamine dyes, including but not limited to Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7 and FluorX; BODIPY dyes, including but not limited to BODIPY-FL, BODIPY-TR, BODIPY-TMR, BODIPY-630/650, and BODIPY-650/670; and ALEXA dyes, including but not limited to ALEXA-488, ALEXA-532, ALEXA-546, ALEXA-568, and ALEXA-594; as well as other fluorescent dyes known to those skilled in the art. Electron rich indicator molecules suitable for the present invention include, but are not limited to, aferritin, hemocyanin, and [colliodal] <u>colloidal</u> gold. Alternatively, in less preferred embodiments the target polynucleotides may be labeled by specifically complexing a first group to the polynucleotide. A second group, covalently linked to an indicator molecule and which has an affinity for the first group, can be used to

indirectly detect the target polynucleotide. In such an embodiment, compounds suitable for use as a first group include, but are not limited to, biotin and iminobiotin.

On page 21, please amend the paragraph beginning "In one embodiment, the microarray is an array", as follows:

In one embodiment, the microarray is an array (*i.e.*, a matrix) in which each positions represents a discrete binding site for a product encoded by a gene (*i.e.*, for an mRNA or for a cDNA derived therefrom). For example, the binding site can be a DNA or DNA analog to which a particular RNA can specifically hybridize. The DNA or DNA analog can be, *e.g.*, a [syntehtic] <u>synthetic</u> oligomer, a full length cDNA, a less-than full length cDNA, or a gene fragment.

On page 22, please amend the paragraph beginning "Such DNA sequences can be obtained", as follows:

Such DNA sequences can be obtained, *e.g.*, by polymerase chain reaction (PCR) amplification of gene segments from, *e.g.*, genomic DNA, mRNA (*e.g.*, from RT-PCR) or from cloned sequences. PCR primers are preferably chosen based on known sequences of the genes or cDNA that result in amplification of unique fragments (*i.e.*, fragments that do not share more than 10 bases of contiguous identical sequence with any other fragment on the microarray). Computer programs that are well known in the art are useful in the design of primers with the required specificity and optimal [amplifcation] <u>amplification</u> properties, such as *Oligo* version 5.0 (National Biosciences). Typically, each probe on the microarray will be between about 20 bases and about 50,000 bases, and usually between about 300 bases and about 1,000 bases in length. PCR methods are well known in the art and are described, *e.g.*, by Innis *et al.*, eds., 1990, *PCR Protocols: A Guide to Methods and Applications*, Academic Press, Inc., San Diego, California. As will be apparent to one skilled in the art, controlled robotic systems are useful for isolating and amplifying nucleic acids.

On page 27, please amend the paragraph beginning "Alternatively, the target polynucleotide", as follows:

Alternatively, the target polynucleotide can be present in the specific hybridization sample in an amount or abundance that is within a minimum and a maximum amount or

abundance that might be expected for any one polynucleotide sequence in a real sample. For example, typically amounts or abundances of a polynucleotide sequence in a real sample (*i.e.*, in a sample of polynucleotide molecules extracted from a cell or organism) can be as low as about 0.0001% or as high as about 3% of the poly A+ mRNA extraced from the cell or [organsim] organism. More preferably, the amount or abundance can be as high as about 2%, more preferably about 1% of the poly A+ mRNA extracted from the cell or [organsim] organism. The amount or abundance of the polynucleotide sequence is more preferably no lower than about 0.0003% of the poly A+ mRNA extracted from the cell or organism. Thus, for example, the target polynucleotide can be present in the specific hybridization sample in an amount or abundance that is equal to or approximately equal to the average or mean amount or abundance of polynucleotides in a real sample. For example, in embodiments wherein the target polynucleotide corresponds to a gene or gene transcript of a cell or organism, the abundance or amount of the target polynucleotide in the specific hybridization sample can be equal to or approximately equal to the mean or average abundance or amount of genes or gene transcripts expressed by the cell or organism. Typical values of the mean or average abundance or amount of genes or gene transcripts expressed by a cell or organism are known to those skilled in the art, and generally depend on the identity of the cell or organism from which the gene or gene transcript is derived. For example, typical preferred values may include approximately 0.04% of all polyA+ mRNA extracted from a cell or organism.

On page 32, please amend the paragraph beginning "In a third preferred embodiment of the invention", as follows:

In a third preferred embodiment of the invention, the specific hybridization sample may contain , not only the target polynucleotide sequence, but also other non-target polynucleotide sequences. For example, in those embodiments of the invention wherein the sequence of the target polynucleotide is the sequence of a particular gene or gene transcript of a cell or [organsim] organism, the nucleotide sequences of the polynucleotide molecules in the first, specific hybridization sample may comprise polynucleotide sequences corresponding to both the target polynucleotide and to the other genes or gene transcripts of the cell or organism. Thus, in such a third embodiment, the specific hybridization sample is preferably identical to the non-specific hybridization sample described, above, for the second preferred embodiment of the invention. In particular, the specific hybridization sample in this

third embodiment of the invention is most preferably a polynucleotide sample obtained from a normal or wild type cell or organism that expresses the gene or gene transcript of the target polynucleotide, as well as other genes or gene transcripts, at normal levels for the cell or organism.

On page 34, please amend the paragraph beginning "The polynucleotide molecules of both", as follows:

The polynucleotide molecules of both the specific hybridization sample and the non-specific hybridization sample are preferably detectably labeled. Preferably, the detectable label is a fluorescent label, *e.g.*, by incorporation of nucleotide analogs. Other labels suitable for use in the present invention include, but are not limited to, biotin, imminobiotin, antigens, cofactors, dinitrophenol, lipoic acid, olefinic compounds, detectable polypeptides, electron rich molecules, enzymes capable of generating a detectable signal by action upon a substrate, and radioactive isotopes. Preferred radioactive isotopes include, ^{32}P, ^{35}S, ^{14}C, ^{15}N and ^{125}I, to name a few. Fluorescent molecules suitable for the present invention include, but are not limited to, fluorescein and its derivatives, rhodamine and its derivatives, texas red, 5'-carboxy-fluorescein ("FMA"), 2',7'-dimethoxy-4',5'-dichloro-6-carboxy-fluorescein ("JOE"), N,N,N',N'-tetramethyl-6-carboxy-rhodamine ("TAMRA"), 6'-carboxy-X-rhodamine ("ROX"), HEX, TET, IRD40 and IRD41. Fluroescent molecules that are suitable for the invention further include: cyamine dyes, including but not limited to Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7 and FluorX; BODIPY dyes, including but not limited to BODIPY-FL, BODIPY-TR, BODIPY-TMR, BODIPY-630/650, and BODIPY-650/670; and ALEXA dyes, including but not limited to ALEXA-488, ALEXA-532, ALEXA-546, ALEXA-568, and ALEXA-594; as well as other fluorescent dyes known to those skilled in the art. Electron rich indicator molecules suitable for the present invention include, but are not limited to, aferritin, hemocyanin, and [colliodal] <u>colloidal</u> gold. Alternatively, in less preferred embodiments the target polynucleotides may be labeled by specifically complexing a first group to the polynucleotide. A second group, covalently linked to an indicator molecule and which has an affinity for the first group, can be used to indirectly detect the target polynucleotide. In such an embodiment, compounds suitable for use as a first group include, but are not limited to, biotin and iminobiotin.

On page 38, please amend the paragraph beginning "In the example described above", as follows:

In the example described above, cDNA from the specific hybridization sample will fluoresce green when the fluorophore (*i.e.*, the fluorescein label) is stimulated, and the cDNA from the non-specific hybridization sample will fluoresce red. As a result, when a particular probe (*e.g.*, on the microarray) hybridizes specifically to a particular target polynucleotide (*i.e.*, the target polynucleotide of the specific hybridization sample) the binding site for that probe on the microarray will emit a wavelength characteristic of the fluorescein label (*i.e.*, green). In contrast, when a probe on the microarray cross-hybridizes to other polynucleotides (*i.e.*, from the non-specific hybridization sample) the binding site for the probe on the microarray will emit a wavelength characteristic of both labels. A probe that hybridizes more specifically to the target polynucleotide of the specific hybridization sample will fluoresce with a higher ratio of green to red fluorescence, whereas a probe that hybridizes less specifically to that target polynucleotide will fluoresce with a lower ratio of green to red [fluroescence] <u>fluorescence</u>.

On page 42, please amend the paragraph beginning "Loaded into the memory during operation", as follows:

Loaded into the memory during operation of this system are several software components which are both standard in the art and special to the instant invention. These software components collectively cause the computer system to function according to the methods of the invention (*i.e.*, they will cause the processor to implement the methods of the invention). The software components are typically encoded and stored on computer readable media such as the mass storage component 704. However, one or more of the software components can be encoded and stored on other forms of computer readable media, including, but not limited to, a floppy disk, a CD-ROM or a DAT tape. Software component 710 represents an operating system which is responsible for managing the computer system and its network interconnections. The operating system can be, for example, of the Microsoft Windows™ family, such as Windows 95, Windows 98, Windows 2000 or Windows NT. Alternatively, the operating system can be a Macintosh operating system or a Unix operating system such as LINUX. Software component 711 represents common languages and functions conveniently present in the system to assist programs implementing the methods

specific to the present invention. Languages that can be used to program the analytic methods of the invention include, for example, C, C++ and, less preferably, FORTRAN and JAVA. Most preferably, the methods of the invention are programmed in mathematical software packages which allow symbolic entry of equations and high-level specification of processing, including specific algorithms to be used, thereby freeing a user of the need to procedurally program individual equations and algorithms. Such packages include, *e.g.*, Matlab from Mathworks (Natick, MA), Mathematic from Wolfram Research ([Chapaign] Champaign, Illinois) or S-Plus from Math Soft (Seattle, Washington). Accordingly, software component 712 represents analytic methods of the present invention as programmed in a procedural language or symbolic package. The software components may also include a component 713 containing data, *e.g.*, in a database, used in the analytical methods of the invention. For example, the database component may comprise data representing the amount of binding (*e.g.*, hybridization) of molecules in one or more samples to a probe or probes. Such computer systems can be used to implement and practice the methods of the present invention. In particular, a user can cause execution of the analysis software component 712 of the system so that the processor implements the methods of the invention and thereby evaluates the binding of one or more probes to one or more different target molecules.

On page 47, please amend the paragraph beginning "The selected YER019W oligonucleotides", as follows:

The selected YER019W oligonucleotides, the YGR192C and YLR040C controls and the negative controls were all printed in duplicate on the top and bottom half of three chips, referred to as Chips 978, 979, and 1136, according to the standard inkjet printing techniques of Blanchard (see, *e.g.*, International Patent Publication No. WO 98/41531, published on September 24, 1998; Blanchard *et al.*, 1996, *Biosensors and [Bioeletronics]* *Bioelectronics* *11*:687-690; Blanchard, 1998, in *Synthetic DNA Arrays in Genetic Engineering*, Vol. 20, J.K. Setlow, ed., Plenum Press, New York at pages 111-123). All chips were [hybrdized ovenight] hybridized overnight at 66 °C in 200 µL of hybridization solution consisting of 10 mM Tris pH 7.6, 1 M NaCl, 1% Triton-X-100; 1 µg/µL bovine serum albumin, 0.1 µg/µL sheared herring sperm DNA, 50 pM Cy3-labeled gridline oligonucleotide, and 50 pM Cy5-labeled gridline oligonucleotide. After hybridization, the chips were washed by shaking for 10 seconds at room temperature in 6x SSPE, 0.005% Triton-X-100; and for another 10

seconds at room temperature in 0.06x SSPE. The chips were dried with pressurized air, and scanned using a General Scanning 3000 confocal laser scanner.

EXHIBIT C: MARKED VERSION OF AMENDED CLAIMS
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(as amended October 9, 2001)

19. (Amended) The method of claim 18 wherein the plurality of probes comprises probes in an array of probes,

said array having a support with at least one surface and different probes attached to said surface, [and]

wherein each [probe] of said different probes is attached to the surface of the support in a different location [on said surface].

26. (Amended) The method of claim 25 wherein the plurality of polynucleotide probes comprises polynucleotide probes in an array of polynucleotide probes,

said array having a support with at least one surface and different polynucleotide probes attached to said surface, [and]

wherein each of said different polynucleotide [probe] probes attached to said surface is attached to the surface of the support in a different location [on said surface].

36. (Amended) The method of claim 31 wherein each different polynucleotide molecule [is] in the second sample has a nucleotide sequence different from the target nucleotide sequence.

39. (Amended) The method of claim 38 wherein:

(a) the target polynucleotide corresponds to a gene or gene transcript of a cell or [organsim] organism; and

(b) the second sample comprises a polynucleotide sample from a wild-type strain of the cell or organism,

wherein the wild-type strain of the cell or organism expresses the gene or gene transcript corresponding to the target polynucleotide.

66. (Amended) The method of claim 65 wherein the plurality of polynucleotide probes comprises polynucleotide probes in an array of polynucleotide probes,

said array having a support with at least one surface and different polynucleotide probes attached to said surface, [and]

wherein each [probe] of said different polynucleotide probes attached to said surface is attached to the surface of the support in a different location [on said surface].

74. (Amended) The method of claim 67 wherein the plurality of polynucleotide probes comprises polynucleotide probes in an array of probes,

said array having a support with at least one surface and different polynucleotide probes attached to said surface, [and]

wherein each [probe] of said different polynucleotide probes attached to said surface in the plurality of probes is attached to the surface of the support in a different location [on said surface].